Glutathione *S*-transferase genotypes and stomach cancer in a population-based case–control study in Warsaw, Poland

Qing Lan[a], Wong-Ho Chow[a], Jolanta Lissowska[b], David W. Hein[c], Kenneth Buetow[a], Lawrence S. Engel[a], Butian Ji[a], Witold Zatonski[b] and Nathaniel Rothman[a]

Glutathione *S*-transferases are important in the detoxification of a wide range of human carcinogens. Previous studies have shown inconsistent associations between the *GSTT1* and *GSTM1* null genotypes and stomach cancer risk. We investigated the relationship between these and related genotypes and stomach cancer risk in a population-based case–control study in Warsaw, Poland, where stomach cancer incidence and mortality rates are among the highest in Europe. DNA from blood samples was available for 304 stomach cancer patients and 427 control subjects. We observed a 1.48-fold increased risk for stomach cancer (95% confidence interval 0.97–2.25) in patients with the *GSTT1* null genotype but no evidence of increased risk associated with the *GSTM1*, *GSTM3* or *GSTP1* genotypes. Furthermore, the stomach cancer risk associated with the *GSTT1* null genotype varied by age at diagnosis, with odds ratios of 3.85, 1.91, 1.78 and 0.59 for those diagnosed at ages less than 50, 50–59, 60–69 and 70 years or older, respectively (*P* trend = 0.01). This was due to a shift in the *GSTT1* genotype distribution across age groups among stomach cancer patients only. These results suggest that the *GSTT1* null genotype may be associated with increased risk of stomach cancer. *Pharmacogenetics* 11:655–661 © 2001 Lippincott Williams & Wilkins

Pharmacogenetics 2001, **11**:655–661

Keywords

[a]Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD, USA, [b]Division of Cancer Epidemiology and Prevention, Cancer Center and M. Sklodowska-Curie Institute of Oncology, Warsaw, Poland and [c]Department of Pharmacology and Toxicology, University of Louisville School of Medicine, Louisville, KY, USA

Correspondence to Qing Lan MD PhD, Occupational Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, MSC 7240, 6120 Executive Blvd, EPS 8109, Bethesda, Maryland 20892-7240, USA
Tel: +1 301 435 4706; fax: +1 301 402 1819; e-mail: qingl@mail.nih.gov

Received 9 January 2001
Accepted 17 May 2001

Introduction

Stomach cancer incidence and mortality rates in Warsaw, Poland, are among the highest in Europe (Zatonski *et al*., 1996). Cigarette smoking and a history of stomach cancer in a first-degree relative have been associated with gastric cancer in Warsaw (Chow *et al*., 1999; Lissowska *et al*., 1999). It is possible that differences in carcinogen metabolism may contribute to the risk of stomach cancer in this population.

Glutathione *S*-transferases (GSTs) are a supergene family of enzymes. In humans, there are at least 13 GST enzymes belonging to five families, namely α (GSTA), μ (GSTM), π (GSTP), σ (GSTS) and θ (GSTT) (Mannervik *et al*., 1992). GSTs catalyze a variety of reducing glutathione-dependent reactions with electrophilic substrates, which include active carcinogen metabolites (Hayes and Pulford, 1995). *GSTM1* and *GSTT1* are of particular interest because of their involvement in the detoxification of reactive metabolites resulting from tobacco smoke (Kelsey *et al*., 1997; Jourenkova *et al*., 1998; Jourenkova-Mironova *et al*., 1999). Homozygous deletions in *GSTM1* and *GSTT1* genes cause an absence of GSTM1 and GSTT1 enzyme activities (Seidegard *et al*., 1988; Hayes and Pulford, 1995). Several published studies have evaluated the relationship between *GSTM1* and/or *GSTT1* and the risk of gastric cancer with inconsistent results (Hayes and Pulford, 1995; Deakin *et al*., 1996; Katoh *et al*., 1999; Setiawan *et al*., 2000). For the *GSTP1* gene, *GSTP1*B* and *GSTP1*C*, two variant alleles, have been detected in addition to the wild-type allele *GSTP1*A* (Ali-Osman *et al*., 1997). *GSTP1* has been shown to detoxify active metabolites of polycyclic aromatic hydrocarbons, which are among the main carcinogens in tobacco smoke (Ali-Osman *et al*., 1997). However, a study by Katoh *et al*. (1999) found no association between *GSTP1* and risk of stomach cancer.

In the *GSTM3* gene, the *GSTM3*A* wild-type and *GSTM3*B* variant alleles have been described. The *GSTM3*B* variant allele contains a recognition motif for the YY1 transcription factor, which has been postulated to regulate gene expression (Inskip *et al*., 1995). This suggests that *GSTM3*A* and *GSTM3*B* express different levels of enzyme and that different *GSTM3*

0960–314X

genotypes may confer different rates of carcinogen metabolism, although we are not aware of any reports to date on the relationship between *GSTM3* and stomach cancer risk.

Different GST isoenzymes exhibit overlapping substrate specificities (Hayes and Pulford, 1995). Deficiencies in a given GST isoenzyme may be compensated by other GST isoforms or through alternative metabolic pathways. Therefore, it is important to determine all of the relevant genotypes in a given gene family. The purpose of this study was to examine the relationship between *GSTT1*, *GSTM1*, *GSTM3* and *GSTP1* genotypes and stomach cancer risk, and to determine whether there were any interactions between various combinations of those genotypes and tobacco smoke.

Materials and methods

Data were derived from a population-based case-control study of stomach cancer that was carried out in Warsaw, Poland, between 1994 and 1996. The study population has been described in detail previously (Chow *et al*., 1999). In brief, cases consisted of persons newly diagnosed with stomach cancer between 1 March 1994 and 30 April 1996, who were identified by collaborating physicians in each of the 22 hospitals serving the study. In addition, Cancer Registry files were reviewed regularly to ensure completeness of case ascertainment. Controls were randomly selected from among Warsaw residents using a computerized registry of all legal residents of Poland. They were frequency-matched to cases by gender and by age in 5-year strata. Detailed information on lifetime tobacco use, alcohol consumption, family history of stomach cancer, childhood living conditions and usual diet prior to 1990 was recorded during a personal interview.

Of the 515 eligible patients identified, interviews were conducted in-person for 324 patients (62.9%) and with the next of kin of 140 patients (27.2%). A 30 ml blood sample was collected from 304 patients (63.3%). Tumor samples were collected from most deceased patients, but few could be analyzed for genetic polymorphisms because of the inadequate quality or quantity of their DNA.

Of the 549 controls identified, 480 (87.4%) agreed to be interviewed and 433 (78.9%) agreed to donate a 30 ml blood sample.

Genotyping

DNA extraction was performed using protocols by Daly *et al*. (1996). For detection of *GSTM1**0 homozygotes, the method of Zhong *et al*. (1993) was used. *GSTM3* and *GSTP1* genotypes were determined using polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) methods described by Inskip *et al*. (1995) and Watson *et al*. (1998). The *GSTT1* assay used the method described by Voltz *et al*. (1999; unpublished data), which detects the gene deletion described by Pemble *et al*. (1994) and a SNP defining a T824C transition in the 3′ untranslated region (UTR) of *GSTT1*. DNA samples were genotyped blind to case–control status.

In brief, for the *GSTM1* assay, amplification was carried out using primers 5′-CGCCATCTTGTGCTACA TTGCCCG-3′, 5′-ATCTTCTCCTCTTCTGTCT-3′ and 5′-TTCTGATTGTAGCAGATCA-3′. The PCR involved 30 cycles of 1 min at 94°C, 2 min at 50°C and 2 min at 70°C. Products were analyzed by electrophoresis on a 1% agarose gel. A band of 230 bp indicated the *GSTM1* wild-type allele; this band was missing for patients who were homozygous null (*GSTM1**0). *GSTT1* genotype was determined according to the PCR-RFLP method (Pemble *et al*., 1994). A 350 bp fragment from *GSTT1* was amplified along with a 268 bp fragment from β-globin by PCR using primers 5′-TGTAAAACGACGGCCAGTCCCATGAGGTCAT TCTGAAG-3′ and 5′-CAGGAAACAGCTATGACC TAAAGGACACAAGGCCTCAG-3′, β-globin: 5′-CAA CTTCATCCACGTTCACC-3′ and 5′-GAAGAGCC AAGGACAGTTAC-3′ in a reaction containing 20 ng of genomic DNA, 15 mmol Tris-HCl, 50 mmol KCl, 1.5 mmol $MgCl_2$, 0.8 μm deoxyribonucleoside triphosphate (dNTP) mix, 0.5 μm of each primer and 0.5 U AmpliTaq gold DNA polymerase. PCR cycling conditions were three cycles of 94°C for 30 s, 62°C for 30 s and 72°C for 30 s, followed by three cycles of 94°C for 30 s, 60°C for 30 s and 72°C for 30 s, and then followed by 30 cycles of 94°C for 30 s, 58°C for 30 s and 72°C for 30 s. The PCR products were treated with Mse1 restriction endonuclease at 37°C for 2 h (NEB), and electrophoreses on a 4% Seakem agarose gel in 1X TAE for 3 h at 70 V to detect the T824C transition in the 3′ UTR of *GSTT1*. The C/C homozygote yielded a band at 350 bp, while the T/T homozygote yielded bands sized 215 bp and 135 bp. A β-globin-specific fragment was used as a positive control to confirm that the PCR reaction had worked properly. The presence of a 268 bp fragment corresponding to β-globin and the absence of the 350 bp product signified a null allele.

The *GSTM3* genotype was determined according to the PCR-RFLP method described by Inskip *et al*. (1995). First, a 273 bp fragment spanning exons 6 and 7 of *GSTM3* was amplified using the primers 5′-CCTCAG TACTTGGAAGAGCT-3′ and 5′-CACATGAAAGCC TTCAGGTT-3′. The PCR products were treated with Mnl I restriction endonuclease at 37°C for 2 h (NEB) and then electrophoresed on a 4% agarose gel in 1X TAE for 4 h at 60 V in order to detect the 3 bp deletion in intron 6. The 273 bp fragment was then digested into four fragments of sizes 125 bp, 86 bp, 51 bp and

11 bp; the 270 bp fragment (containing the three nucleotide deletions) was digested into three fragments of sizes 134 bp, 125 bp and 11 bp. *GSTP1* genotype was determined according to the PCR-RFLP method of Watson *et al.* (1998). Briefly, a 329 bp fragment of exon 5 was amplified by PCR using primers: 5′-GTAGTTTGCCCAAGGTCAAG-3′ and 5′-AGCCA CCTGAGGGGTAAG-3′ in a reaction containing 1 μg genomic DNA, 10 mmol Tris-HCI (pH 8.3), 50 mmol KCl, 1.5 mmol $MgCl_2$, 0.2 mmol of each dNTP, 0.5 μg primer, and 1.2 U Taq DNA polymerase. The PCR product was digested with 5 U Alw261 at 37°C for 3 h in order to detect the G313A. Ile105 homozygotes produce bands at 329 bp and 113 bp; Val105 homozygotes yield bands at 216 bp, 113 bp and 107 bp; and Ile/Val heterozygotes yield bands at 329 bp, 216 bp, 113 bp and 107 bp.

Data were available from blood samples for 289 patients for the *GSTM1* genotype, 279 patients for the *GSTT1* genotype, 290 patients for the *GSTP1* genotype and 278 patients for the *GSTM3* genotype. Data were available from tumor samples for 58 patients for *GSTM1*, 14 patients for *GSTT1*, 10 patients for *GSTP1* and 14 patients for *GSTM3*. Data were available from blood samples on 426 control subjects for *GSTM1*, 418 control subjects for *GSTT1*, 421 control subjects for *GSTP1* and 417 control subjects for *GSTM3*. Subsequent statistical analyses, restricted to patients whose DNA sample came from blood, gave results that were very similar to analyses that used all the available data; here, we present results using all data.

Statistical analysis

Odds ratios and 95% confidence intervals, which were used to estimate the association between stomach cancer and *GST* genotypes and other risk factors, were calculated via unconditional logistic regression using SAS 6.12 (SAS Institute Inc., Cary, NC, USA). Odds ratios adjusted for known or suspected stomach cancer risk factors (i.e. age, gender, education, pack-years of cigarette smoking, family history of stomach cancer, years lived on a farm and fruit intake) were similar to odds ratios adjusted for only age and gender. Because further adjustment for *Helicobactor pylori* infection, body mass index (BMI) and interleukin-1 polymorphisms had no important effect on results, we did not adjust for these in the analysis presented here.

Pack-years of cigarette smoking were computed by multiplying the number of packs of cigarettes smoked per day by the total years of smoking. Gene–gene and gene–smoking multiplicative interactions were evaluated by the likelihood ratio test, comparing the goodness of fit of the model with and without the interaction term. Interactions between *GST* genotypes and family history of stomach cancer could not be evaluated because of small numbers. We tested for an interaction between *GSTT1* genotype and age by evaluating the age trend in the *GSTT1* effect. To accomplish this, we created a variable that had a zero value for all patients except for *GSTT1* nulls aged 21–49 years, 50–59 years, 60–69 years and ⩾ 70 years, who were assigned values 1, 2, 3 and 4 respectively.

Results

In order to assess potential selection bias due to differential availability of blood samples, we compared selected demographic and lifestyle characteristics in subjects who provided blood samples with those in subjects who did not (Table 1). There were no statistically significant differences between patients with or without blood samples for either stomach cancer patients or control subjects. Patients with blood samples tended to have slightly higher BMIs than those without blood samples. Control subjects with blood samples tended to have fewer pack-years of cigarette smoking than control subjects without blood samples.

Odds ratios for stomach cancer associated with *GST* genotypes are presented in Table 2. The *GSTT1* null genotype was associated with a borderline significant increase in risk of stomach cancer (odds ratio 1.48; 95% confidence interval 0.97–2.25). We observed no association with the other genotypes examined, although risk was reduced among those with *GSTP1* mutant alleles (Table 2). We found no evidence for significant interactions between the different *GST* genotypes and risk of stomach cancer (data not shown). Among stomach cancer patients, we observed no significant relationship between the *GSTT1* null genotype and tumor grade ($\chi^2 = 3.05$; $P = 0.81$; data available for 152 patients), stage ($\chi^2 = 2.49$; $P = 0.29$; data available for 225 patients), tumor site (i.e. cardia, distal and combined: $\chi^2 = 0.67$; $P = 0.72$; data available for 282 patients) or Lauren classification (i.e. intestinal, diffuse and indeterminate: $\chi^2 = 2.13$, $P = 0.35$; data available for 276 patients).

The risk associated with the *GSTT1* null genotype varied with age at diagnosis (Table 3). This is consistent with the clear trend of decreasing *GSTT1* null genotype prevalence with increasing age among the stomach cancer patients but not among the control subjects. Similar results were obtained after stratification by tumor grade, stage, site and Lauren classification groups (data not shown).

Table 4 shows the *GSTT1* genotype by smoking status. There was some suggestion that current smoking was more strongly associated with stomach cancer among *GSTT1* null patients than among *GSTT1* positive patients. Compared to *GSTT1* positive non-smokers (odds

Table 1 **Distribution of selected variables in gastric cancer cases and controls by blood sample availability**

	Gastric cancer *n* (%)		Control subjects *n* (%)	
	With sample $n = 304$	Without sample $n = 160$	With sample $n = 427$	Without sample $n = 53$
Age (years)				
21–50	39 (12.8)	17 (10.6)	52 (12.2)	7 (13.2)
50–59	54 (17.8)	21 (13.1)	74 (17.3)	10 (18.9)
60–69	119 (39.1)	57 (35.6)	169 (39.6)	16 (30.2)
70–79	92 (30.3)	65 (40.6)	132 (30.9)	20 (37.7)
χ^2 *P* value	0.14		0.60	
Gender				
Male	200 (65.8)	102 (63.8)	275 (64.4)	39 (73.6)
Female	104 (34.2)	58 (36.3)	152 (35.6)	14 (26.4)
χ^2 *P* value	0.66		0.19	
Education				
Less than high school	144 (47.4)	62 (38.8)	160 (37.5)	23 (43.4)
High school or technical training	99 (32.6)	57 (35.6)	130 (30.4)	18 (34.0)
Some college/college graduate	61 (20.1)	41 (25.6)	137 (32.1)	12 (22.6)
χ^2 *P* value	0.17		0.37	
Smoking status				
Never	84 (27.9)	54 (36.7)	171 (40.1)	14 (26.9)
Ex-smokers	123 (40.9)	49 (33.3)	143 (33.5)	22 (42.3)
Current smokers	94 (31.2)	44 (29.9)	113 (26.5)	16 (30.7)
χ^2 *P* value	0.14		0.18	
Cigarette smoking (pack-years)				
0	84 (28.0)	54 (34.6)	171 (40.1)	14 (27.5)
20 ⩽ 40	59 (19.7)	23 (14.7)	83 (19.5)	8 (15.7)
⩾ 40	157 (52.3)	79 (50.6)	172 (40.4)	29 (56.9)
χ^2 *P* value	0.23		0.08	
Fruit intake				
Daily or several times/week	133 (46.3)	49 (47.1)	206 (48.4)	29 (55.8)
Several times/month	99 (35.2)	30 (28.9)	141 (33.1)	17 (32.7)
Few times/year or never	52 (18.5)	25 (24.0)	79 (18.5)	6 (7.1)
χ^2 *P* value	0.35		0.41	
Body mass index (kg/m^2)				
< 23.1	64 (21.1)	42 (26.3)	107 (25.1)	14 (26.4)
23.1–25.4	84 (27.6)	42 (26.3)	108 (25.3)	14 (26.4)
25.5–28.1	79 (26.0)	50 (31.3)	110 (25.8)	9 (17.0)
> 28.1	77 (25.3)	26 (16.3)	102 (23.9)	16 (30.2)
χ^2 *P* value	0.10		0.52	

Table 2 ***GSTM1, GSTT1, GSTP1*** **and** ***GSTM3*** **genotypes and the risk of stomach cancer**

Genotypes[a]	Cases	Control subjects	Odds ratio[b]	95% confidence interval	Odds ratio[c]	95% confidence interval
GSTM1						
Positive	180	204	1.00	reference	1.00	reference
Null	167	222	0.85	(0.64–1.13)	0.92	(0.67–1.26)
GSTT1						
Positive	233	352	1.00	reference	1.00	reference
Null	60	66	1.37	(0.93–2.02)	1.48	(0.97–2.25)
GSTP1						
AA	142	177	1.00	reference	1.00	reference
AG	133	202	0.82	(0.60–1.12)	0.74	(0.52–1.04)
GG	25	42	0.74	(0.43–1.27)	0.67	(0.37–1.20)
GSTM3						
AB or BB	74	108	1.00	reference	1.00	reference
AA	218	309	1.03	(0.73–1.46)	0.99	(0.68–1.44)

[a]number of cases genotyped from tumor DNA: *GSTM1* 58; *GSTT1* 14; *GSTP1* 10; *GSTM3* 14; [b]adjusted for age and gender; [c]adjusted for age, gender, education, tobacco smoke, years lived on a farm, fruit intake and family history of stomach cancer.

ratio = 1.0), odds ratios for ex-smokers and current smokers among *GSTT1* positive patients were 1.75 and 1.54, respectively, while among *GSTT1* null patients, odds ratios for non-smokers, ex-smokers and current smokers were 1.18, 2.27 and 3.08, respectively. However, no significant interactions were observed between *GSTT1* genotype and smoking status, pack-years of smoking or age at which the patient started smoking.

Discussion

We carried out a population-based case–control study of stomach cancer in Warsaw, Poland, and observed a

Table 3 ***GSTT1*** **genotype and risk of stomach cancer by age at diagnosis**

Age	Cases		Control subjects		Odds ratio[a] (95% confidence interval)	Odds ratio[b] (95% confidence interval)
	Positive *n* (%)	Null *n* (%)	Positive *n* (%)	Null *n* (%)		
21–49	24 (66.7)	12 (33.3)	44 (86.3)	7 (13.7)	3.04 (1.05–8.80)	3.85 (0.87–11.11)
50–59	39 (75.0)	13 (25.0)	63 (86.3)	10 (13.7)	2.20 (0.87–5.57)	1.91 (0.65–5.62)
60–69	91 (78.5)	25 (21.6)	138 (85.2)	24 (14.8)	1.70 (0.91–3.20)	1.78 (0.89–3.54)
⩾ 70	79 (88.8)	10 (11.2)	107 (81.1)	25 (18.9)	0.52 (0.23–1.17)	0.59 (0.23–1.49)
Total	233	60	352	66		
		$P = 0.03$[c]		$P = 0.66$[c]	P for trend $= 0.009$[d]	P for trend $= 0.01$[d]

[a]Adjusted for age and gender; [b]adjusted for age, gender, education, tobacco smoke, years lived on a farm, fruit intake and family history of stomach cancer; [c]χ^2 test for difference in distribution between *GSTT1* positive and null patients; [d]trend for interaction of *GSTT1* with increasing age was assessed via a grouped linear variable in which the group values were assigned in increasing order as follows: (1) all *GSTT1* positive patients; (2) *GSTT1* null patients: aged <50; 50–59; 60–69; and ⩾ 70 years. For interaction between *GSTT1* and age expressed as a continuous variable, $P = 0.03$.

Table 4 **Stomach cancer risk in relation to** ***GSTT1*** **genotype and tobacco smoking status[a]**

GSTT1 and smoking status	Cases	Control subjects	Odds ratio[b]	95% confidence interval	Odds ratio[c]	95% confidence interval
GSTT1 positive						
Non-smoker	66	139	1.00	Reference	1.00	Reference
Ex-smoker	98	120	1.84	(1.21–2.81)	1.75	(1.07–2.86)
Current smoker	67	93	1.62	(1.02–2.56)	1.54	(0.94–2.53)
GSTT1 null						
Non-smoker	17	30	1.19	(0.61–2.31)	1.18	(0.58–2.40)
Ex-smoker	19	20	2.15	(1.06–4.37)	2.27	(1.05–4.89)
Current smoker	24	16	3.35	(1.63–6.85)	3.08	(1.45–6.54)

[a]Interaction between *GSTT1* genotype and smoking status, $P = 0.32$; [b]adjusted for age and gender; [c]adjusted for age, gender, education, tobacco smoke, years lived on a farm, fruit intake and family history of stomach cancer.

borderline significant increased risk for stomach cancer associated with the *GSTT1* null genotype. We found no association between stomach cancer risk and either *GSTM1* or *GSTM3* genotypes.

The findings of the three published studies on *GSTT1* null genotype and stomach cancer are inconsistent, with two reporting no association and one reporting increased risk (Table 5). Deakin *et al.* (1996) reported no association among 114 stomach cancer patients and 509 control subjects in a multi-cancer (lung, oral, gastric and colorectal) hospital-based study with both case and control samples collected from a hospital in the United Kingdom. Katoh *et al.* (1996) carried out a hospital-based case-control study in Japan and reported no increased risk associated with the *GSTT1* null genotype among the 139 stomach cancer patients and 126 control subjects (odds ratio = 1.13; 95% confidence interval 0.70–1.83). In contrast, a recent population-based case–control study of gastric cancer in China (Setiawan *et al.*, 2000) observed an odds ratio for the *GSTT1* null genotype of 2.50 (95% confidence interval 1.01–6.22).

Several limitations of the hospital-based studies may have contributed to the observed heterogeneity of the results. Neither study accounted for confounders such as smoking and age in the risk estimates. Also, the selection of control subjects may have biased risk estimates since diseases exhibited by hospital- or clinic-based control patients may be related to *GSTT1* genotype. Diseases reported to be associated with the *GSTT1* genotype include senile cortical cataract (Juronen *et al.*, 2000), asthma and tuberculosis (Kim *et al.*, 1998).

The results of this study suggest that the effect of *GSTT1* on stomach cancer risk varied with age, with the greatest relative risks at younger ages. This was due to a gradient of decreasing *GSTT1* null genotype prevalence with increasing age among stomach cancer patients, but not among control subjects. Although we are unaware of any other studies reporting this relationship between *GSTT1* and age at diagnosis of stomach cancer, this finding is in agreement with several studies of other cancer sites. Chenevix-Trench *et al.* (1995) reported that *GSTT1* null homozygotes were significantly more common among patients with sporadic colorectal cancer diagnosed before 70 years of age than among those with cancer diagnosed at older ages. Other case–control studies of colorectal cancer (Welfare *et al.*, 1999) and head and neck carcinoma (Cheng *et al.*, 1999) found that the *GSTT1* null genotype was less common with advancing age in both cancer patients and control subjects, but the age gradient was stronger in cancer patients than in control subjects. There is an alternative explanation: a fairly modest elevation, consistent across age, in absolute risk for stomach cancer due to the *GSTT1* null genotype may be obscured on the relative risk scale by the increasing risk among *GSTT* non-null subjects with age.

Table 5 **Summary of published studies of *GSTT1* null genotype and stomach cancer**

Study	Location	Study design	Case definition	Number of cases	Number of control subjects	Percentage of control subjects (*GSTT1* null)	Crude odds ratio[a] (95% confidence interval)	Adjusted odds ratio (95% confidence interval)
Deakin *et al*., 1996	North Staffordshire, UK	Hospital-based	Gastric cancer	114	509	18.5	1.00 (0.59–1.68)	Not applicable
Katoh *et al*., 1996	Kitakyushu, Japan	Hospital-based	Gastric adenocarcinoma	139	126	44.4	1.13 (0.70–1.83)	Not applicable
Setiawan *et al*., 2000	Jiangsu Province China	Population-based	Gastric cancer	81	418	45.5	1.43 (0.89–2.30)	2.50[b] (1.01–6.22)
This study	Warsaw, Poland	Population-based	Gastric cancer	293	418	15.8	1.37 (0.93–2.02)	1.48[c] (0.97–2.25)

[a]Unadjusted; [b]adjusted for age, education, body mass index, pack-years of smoking, fruit intake, salt intake, *Helicobacter pylori* and alcohol drinking; [c]adjusted for age, gender, education, tobacco smoke, years lived on a farm and family history of stomach cancer.

Our study also suggested a possible protective effect of the *GSTP1* mutant allele in relation to stomach cancer (Table 2). However, this association was in the opposite direction of our *a priori* hypothesis, and should be examined in future studies.

To the best of our knowledge, this is the largest study to date to examine *GSTT1*, *GSTM1*, *GSTM3* and *GSTP1* genotypes in relation to stomach cancer. The fact that this study was population-based and had high participation rates strengthens its findings. However, this study does have several limitations. Despite its size, the number of patients in the subanalyses was small, resulting in limited power for those analyses. In addition, 27% of cancer patients died before interview or phlebotomy, mostly due to advanced disease. If *GST* genotypes are related to survival, then our results might not be generally applicable to deceased cases. However, the analyses showed no significant relationship between *GSTT1* genotype and tumor grade or stage, factors that may influence survival.

The GSTs, a superfamily of detoxification enzymes with overlapping substrate specificities, are involved in detoxifying a wide variety of potentially carcinogenic compounds (Ketterer, 1988). In particular, the human θ-class GSTs display activity against a broad range of compounds, including methyl halides, ethylene oxide, 1,2-propylene oxide compounds, 1,3-butadiene and sulfate esters (Dunkelberg *et al*., 1982; Hallier *et al*., 1990; Meyer *et al*., 1991; Thier *et al*., 1991; Schroder *et al*., 1992; Hallier *et al*., 1993, 1994; Warholm *et al*., 1994; Ploemen *et al*., 1995). However, it is not clear which specific chemicals are related to stomach cancer risk. Further studies are needed to confirm the association between *GSTT1* null genotype and stomach cancer risk and to elucidate the specific role that the *GSTT1* genotype may play in the pathogenesis of stomach cancer.

In summary, we observed a borderline significant association between the *GSTT1* null genotype and increased risk of stomach cancer. We also found that the association varied with age. The *GSTM1*, *GSTM3* and *GSTP1* genotypes were not significantly associated with stomach cancer risk in our study.

Acknowledgements

The study was partially supported by USPHS grant CA-34627.

References

Ali-Osman F, Brunner JM, Kutluk TM, Hess K. Prognostic significance of glutathione S-transferase pi expression and subcellular localization in human gliomas. *Clin Cancer Res* 1997; **3**:2253–2261.

Chenevix-Trench G, Young J, Coggan M, Board P. Glutathione S-transferase

M1 and T1 polymorphisms: susceptibility to colon cancer and age of onset. *Carcinogenesis* 1995; **16:**1655–1657.

Cheng L, Sturgis EM, Eicher SA, Char D, Spitz MR, Wei Q. Glutathione-S-transferase polymorphisms and risk of squamous-cell carcinoma of the head and neck. *Int J Cancer* 1999; **84:**220–224.

Chow WH, Swanson CA, Lissowska J, Groves FD, Sobin LH, Nasierowska-Guttmejer A *et al*. Risk of stomach cancer in relation to consumption of cigarettes, alcohol, tea and coffee in Warsaw, Poland. *Int J Cancer* 1999; **81:**871–876.

Daly AK, Steen VM, Fairbrother KS, Idle JR. CYP2D6 multi-allelism. *Methods Enzymol* 1996; **272:**199–210.

Deakin M, Elder J, Hendrickse C, Peckham D, Baldwin D, Pantin C *et al*. Glutathione S-transferase *GSTT1* genotypes and susceptibility to cancer: studies of interactions with *GSTM1* in lung, oral, gastric and colorectal cancers. *Carcinogenesis* 1996; **17:**881–884.

Dunkelberg H. Carcinogenicity of ethylene oxide and 1,2-propylene oxide upon intragastric administration to rats. *Br J Cancer* 1982; **46:**924–933.

Hallier E, Deutschmann S, Reichel C, Bolt HM, Peter H. A comparative investigation of the metabolism of methyl bromide and methyl iodide in human erythrocytes. *Int Arch Occup Environ Health* 1990; **62:**221–225.

Hallier E, Langhof T, Dannappel D, Leutbecher M, Schroder K, Goergens HW *et al*. Polymorphism of glutathione conjugation of methyl bromide, ethylene oxide and dichloromethane in human blood: influence on the induction of sister chromatid exchanges (SCE) in lymphocytes. *Arch Toxicol* 1993; **67:**173–178.

Hallier E, Schroder KR, Asmuth K, Dommermuth A, Aust B, Goergens HW. Metabolism of dichloromethane (methylene chloride) to formaldehyde in human erythrocytes: influence of polymorphism of glutathione transferase theta (GST T1-1). *Arch Toxicol* 1994; **68:**423–427.

Hayes JD, Pulford DJ. The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. *Crit Rev Biochem Mol Biol* 1995; **30:** 445–600.

Inskip A, Elexperu-Camiruaga J, Buxton N, Dias PS, MacIntosh J, Campbell D *et al*. Identification of polymorphism at the glutathione S-transferase, GSTM3 locus: evidence for linkage with GSTM1*A. *Biochem J* 1995; **312:**713–716.

Jourenkova-Mironova N, Voho A, Bouchardy C, Wikman H, Dayer P, Benhamou S, Hirvonen A. Glutathione S-transferase *GSTM1*, *GSTM3*, *GSTP1* and *GSTT1* genotypes and the risk of smoking-related oral and pharyngeal cancers. *Int J Cancer* 1999; **81:**44–48.

Jourenkova N, Reinikainen M, Bouchardy C, Dayer P, Benhamou S, Hirvonen A. Larynx cancer risk in relation to glutathione S-transferase M1 and T1 genotypes and tobacco smoking. *Cancer Epidemiol Biomarkers Prev* 1998; **7:**19–23.

Juronen E, Tasa G, Veromann S, Parts L, Tiidla A, Pulges R *et al*. Polymorphic glutathione S-transferases as genetic risk factors for senile cortical cataract in Estonians. *Invest Ophthalmol Vis Sci* 2000; **41:**2262–2267.

Katoh T, Kaneko S, Takasawa S, Nagata N, Inatomi H, Ikemura K *et al*. Human glutathione S-transferase P1 polymorphism and susceptibility to smoking related epithelial cancer; oral, lung, gastric, colorectal and urothelial cancer. *Pharmacogenetics* 1999; **9:**165–169.

Katoh T, Nagata N, Kuroda Y, Itoh H, Kawahara A, Kuroki N *et al*. Glutathione S-transferase M1 (*GSTM1*) and T1 (*GSTT1*) genetic polymorphism and susceptibility to gastric and colorectal adenocarcinoma. *Carcinogenesis* 1996; **17:**1855–1859.

Kelsey KT, Spitz MR, Zuo ZF, Wiencke JK. Polymorphisms in the glutathione S-transferase class mu and theta genes interact and increase susceptibility to lung cancer in minority populations (Texas, United States). *Cancer Causes Control* 1997; **8:**554–559.

Ketterer B. Protective role of glutathione and glutathione transferases in mutagenesis and carcinogenesis. *Mutat Res* 1988; **202:**343–361.

Kim YC, Park KO, Kern JA, Park CS, Lim SC, Jang AS, Yang JB. The interactive effect of *Ras*, *HER2*, *P53* and *Bcl-2* expression in predicting the survival of non-small cell lung cancer patients. *Lung Cancer* 1998; **22:**181–190.

Lissowska J, Groves FD, Sobin LH, Fraumeni JF Jr., Nasierowska-Guttmejer A, Radziszewski J *et al*. Family history and risk of stomach cancer in Warsaw, Poland. *Eur J Cancer Prev* 1999; **8:**223–227.

Mannervik B, Awasthi YC, Board PG, Hayes JD, Di Ilio C, Ketterer B *et al*. Nomenclature for human glutathione transferases [letter]. *Biochem J* 1992; **282:**305–306.

Meyer DJ, Coles B, Pemble SE, Gilmore KS, Fraser GM, Ketterer B. Theta, a new class of glutathione transferases purified from rat and man. *Biochem J* 1991; **274:**409–414.

Pemble S, Schroeder KR, Spencer SR, Meyer DJ, Hallier E, Bolt HM *et al*. Human glutathione S-transferase theta (GSTT1): cDNA cloning and the characterization of a genetic polymorphism. *Biochem J* 1994; **300:** 271–276.

Ploemen JH, Wormhoudt LW, van Ommen B, Commandeur JN, Vermeulen NP, van Bladeren PJ. Polymorphism in the glutathione conjugation activity of human erythrocytes towards ethylene dibromide and 1,2-epoxy-3-(p-nitrophenoxy)-propane. *Biochim Biophys Acta* 1995; **1243:**469–476.

Schroder KR, Hallier E, Peter H, Bolt HM. Dissociation of a new glutathione S-transferase activity in human erythrocytes. *Biochem Pharmacol* 1992; **43:**1671–1674.

Seidegard J, Vorachek WR, Pero RW, Pearson WR. Hereditary differences in the expression of the human glutathione transferase active on trans-stilbene oxide are due to a gene deletion. *Proc Natl Acad Sci USA* 1988; **85:**7293–7297.

Setiawan VW, Zhang ZF, Yu GP, Li YL, Lu ML, Tsai CJ *et al*. *GSTT1* and *GSTM1* null genotypes and the risk of gastric cancer: a case-control study in a Chinese population. *Cancer Epidemiol Biomarkers Prev* 2000; **9:** 73–80.

Thier R, Foest U, Deutschmann S, Schroeder KR, Westphal G, Hallier E, Peter H. Distribution of methylene chloride in human blood. *Arch Toxicol Suppl* 1991; **14:**254–258.

Voltz AK, Cassidy A, Cleverly K, Edmonson M, Gandolph M, Harkins E *et al*. Discovery of single nucleotide polymorphisms (SNPs) in biochemical pathway genes as risk factors for cancer. *Am J Hum Gene* 1999; **65(Suppl):**2391.

Warholm M, Alexandrie AK, Hogberg J, Sigvardsson K, Rannug A. Polymorphic distribution of glutathione transferase activity with methyl chloride in human blood. *Pharmacogenetics* 1994; **4:**307–311.

Watson MA, Stewart RK, Smith GB, Massey TE, Bell DA. Human glutathione S-transferase P1 polymorphisms: relationship to lung tissue enzyme activity and population frequency distribution. *Carcinogenesis* 1998; **19:**275–280.

Welfare M, Monesola AA, Bassendine MF, Daly AK. Polymorphisms in *GSTP1*, *GSTM1*, and *GSTT1* and susceptibility to colorectal cancer. *Cancer Epidemiol Biomarkers Prev* 1999; **8:**289–292.

Zatonski W, Smans M, Tyczynski J, Boyle P. Atlas of cancer mortality in central Europe. *IARC Scientific Publications* 134; 1996; **134:**1–175.

Zhong S, Wyllie AH, Barnes D, Wolf CR, Spurr NK. Relationship between the *GSTM1* genetic polymorphism and susceptibility to bladder, breast and colon cancer. *Carcinogenesis* 1993; **14:**1821–1824.